Binding partners for 5-HT5 receptors for migraine treatment

The present invention relates to binding partners for 5-HT5 receptors, processes for the identification and characterization of binding partners of this type, and also pharmaceutical compositions comprising them and their use for the treatment of cerebrovascular disorders such as migraine.

At least seven different receptor classes mediate the manifold physiological activities which are ascribed to an involvement of the neurotransmitter serotonin (5-hydroxytryptamine, abbreviated 5-HT). According to an internationally recognized classification, they are designated by 5-HT1, 5-HT2, 5-HT3, 5-HT4, 5-HT5, 5-HT6 and 5-HT7. Most of these classes moreover include receptor types which can be differentiated further. Thus the 5-HT1 class includes receptors which can be divided into at least five subclasses, which are designated by 5-HT1A, 5-HT1B, 5-HT1C, 5-HT1D and 5-HT1E (Boess F.G. and Martin I.L., Neuropharmacology 33:275-317 (1994)).

The 5-HT5 class was described for the first time by Plassat et al., The EMBO Journal Vol. 11 No. 13, pp. 4779-4786 (1992). 5-HT5A and 5-HT5B receptors are differentiated (Erlander et al., Proc. Natl. Acad. Sci. USA 90:3452-3456 (1993)). Only small sequence homologies exist between 5-HT5 and other 5-HT receptors. The pharmacological profile of these receptors differs markedly. Using molecular biology techniques, the localization of 5-HT5 receptors was possible in the olfactory bulb, in the hippocampus, in the cortex, in the cerebral ventricles, in the corpus callosum and in the cerebellum. By means of immunohistochemical methods, it was possible to show that 5-HT5 receptors are principally expressed on astrocytes (Carson et al., GLIA 17:317-326 (1996)). Astrocytes are directly adjacent to the basal membrane of brain capillaries of the blood-brain barrier. An abnormal astrocyte endothelium structure accompanies a loss of the blood-brain barrier. The exact significance of the astrocytes is unclear. They appear to carry out transport tasks and connective functions. Reactive astrocytes were observed in connection with reactive gliosis in a number of pathological brain changes and neuropsychiatric disorders. As a result of brain injuries, they change their morphologies. The protein expression pattern changes and growth factors are produced. In vitro investigations on cultured astrocytes have allowed the detection of 5-HT5 receptor-mediated responses. It is thus to be suspected on the one hand that they are involved in recovery processes of the brain after disorders, but on the other hand it is also not to be

excluded that they contribute to the creation of damage or even to an increase in damage.

Migraine is a CNS disorder which affects larg parts of the population. In most cases, it is manifest d by headaches which recur again and again, by which, at a rough estimate, 8 million people, i.e. 3-5% of all children, 7% of all men and 14% of all women, are affected. Even though a genetic predisposition is propagated, the causes appear to be complex (Diener H.C. et al., Arzneimitteltherapie 15:387-394 (1997)).

Two hypotheses are dominant. The already long-known vascular theory proposes as a cause a dilatation process of the internal and external cerebral vascular system. The neurogenic theory is based on secretion of vasoactive neurotransmitters, principally neuropeptides, such as substance P and neurokinin, from axons of the vasculature as a result of stimulation of certain ganglia which innervate brain tissue, and which should lead to inflammatory reactions and thus to pain.

At present, there is still no causal therapy for the treatment of migraine. Two different treatment methods are used at present, a first, prophylactic therapy for the prevention of recurring migraine attacks and a second, symptomatic therapy for the suppression of acute symptoms during attacks. Migraine-specific active compounds, such as Sanmigran®, Nocerton®, Desernil® and Vidora®, but also active compounds usually used for other indications, such as beta-blockers, antiemetic active compounds, such as Sibelium®, antidepressants such as Laroxyl®, or antiepileptic active compounds such as Depakin®, are administered prophylactically. In the course of acute therapy, analgesics are given, such as aspirin, paracetamol or Optalidon®, nonsteroidal antiinflammatories, such as Cebutid®, Voltaren®, Brufen®, Ponstyl®, Profenid®, Apranx® and Naprosin® against pain and inflammation, ergot alkaloids, such as ergotamine, dihydroergotamine, which can induce vasoconstriction, or substances of the triptan family, such as sumatriptan, Naramig®, and AscoTop® having a high affinity for 5-HT1D receptors. The latter substances act as agonists and block vasodilatation.

The active compounds mentioned, however, are not optimally suited for the treatment of migraine. Nonopioid analgesics often have side effects. As a result of the strong peripheral vasoconstriction, the complex mechanism of action of the ergot alkaloids leads to side effects such as hypertension and gangrene. Compounds belonging to the triptan family are also not

completely satisfactory (Pfaffenrath V., Münch. med. Wschr. 625-626 (1998)).

Sumatriptan (Imigran®), one of the most effective and most frequently employed active compounds against acute migraine attacks, does not pass through the blood-brain barrier on account of marked hydrophilicity. In 28% of the patients, this active compound is ineffective and the oral doses of 50-100 mg are quite high. Contraindications which have been known are coronary vasospasms, hypertension, kidney and liver disorders.

Up to now, compounds having selective affinity for 5-HT1 receptors have been considered for the treatment of migraine (Goadsby P.J., CNS Drugs 10:271-286 (1998); Saxena P.R., Exp. Opin. Invest. Drugs 581-593 (1996)). For example, sumatriptan counts as a very selective ligand for 5-HT1D receptors, which is why the professional world are at pains to optimize these binding properties and the vasoconstrictive activities mediated thereby. The same applies to 5-HT1B and 5-HT1F receptor ligands. Thus, a series of active compounds was developed which, however, were also unable to remedy the problems outlined.

It is therefore an object of the present invention to make possible the preferably acute tratment of migraine-like cerebrovascular disorders with adequate efficacy and slight side effects.

Surprisingly, it has now been found that substances having comparatively high binding affinity for 5-HT5 receptors can provide the effects desired.

The present invention therefore relates to selective binding partners for 5-HT5 receptors, whose binding affinity for 5-HT5 receptors is greater than for one or more 5-HT receptors other than 5-HT5.

The term "binding partner for 5-HT5 receptors" describes substances which bind to 5-HT5 receptors and can therefore also be designated as 5-HT5 receptor ligands.

or include metal complex-like coordinative bonds. [sic] In addition to the abovementioned, reversible molecular interactions, irreversible interactions between binding partner and receptor can also be possible, such as, for example, covalent bonds.

Selectivity is understood as meaning the property of a binding partner to bind preferably to 5-HT5 receptors.

Thus binding partners have binding affinities for 5-HT5 receptors which are greater than for one or mor 5-HT receptors other than 5-HT5, i.e. in particular receptors to be assigned to the abovementioned 5-HT receptor classes 5-HT1, 5-HT2, 5-HT3, 5-HT4, 5-HT6 and 5-HT7. If the binding affinity for 5-HT5 receptors of a binding partner is greater than that of a 5-HT receptor other than 5-HT5, in relation to the 5-HT receptor which is other than 5-HT5 a more selective binding of these binding partners to 5-HT5 receptors is referred to. Particular binding partners are those whose binding affinity for 5-HT5 receptors is greater than for at least one and, in particular, all 5-HT1 receptors, in particular for 5-HT1D and/or 5-HT1B receptors. Binding partners whose binding affinity for 5-HT5 receptors is greater than for all 5-HT receptors other than 5-HT5 are a further particular class of binding partners according to the invention.

It is decisive for the selectivity outlined above that the binding affinities for 5-HT5 receptors, on the one hand, and for one or more 5-HT receptors other than 5-HT5, on the other hand, differ sufficiently. Affinity differences are preferred whereby binding affinity ratios of at least 2, more advantageously of at least 5, particularly advantageously of at least 10, preferably of at least 20, particularly preferably of at least 50 and in particular of at least 100, are present.

According to one embodiment, binding partners according to the invention competitively inhibit the binding of comparison binding partners, such as 5-HT (5-hydroxytryptamine) or 5-CT (5-carboxamidotryptamine), to 5-HT5 receptors.

Competitive inhibition is understood as meaning that binding partners according to the invention compete with a comparison binding partner, in the present, case for example, 5-HT or 5-CT, for binding to the receptor, i.e. the binding of one prevents the binding of the other.

According to a further embodiment, binding partners according to the invention inhibit the binding of comparison binding partners, such as 5-HT (5-hydroxytryptamine) or 5-CT (5-carboxamido-tryptamine), to 5-HT5 receptors noncompetitively.

Noncompetitive inhibition is understood as meaning that binding partners according to the invention modulate the binding of a comparison binding partner, in the present case, for example,

5-HT or 5-CT, in particular d crease its binding affinity, via their binding to the receptor.

At least in the case of competitive inhibition, i.e. of reversible binding, the principle applies that the displacement of one binding partner by another increases with decreasing binding affinity of the one or increasing binding affinity of the other with respect to the receptor. More expediently, therefore, binding partners which can be used according to the invention have a high binding affinity for 5-HT5 receptors. A binding affinity of this type allows, on the one hand, an effective displacement of naturally occurring binding partners for 5-HT5 receptors, such as, for example, serotonin (5-hydroxytryptamine, 5-HT) itself, where the necessary concentration of binding partners which can be used according to the invention for the binding of a certain amount of this binding partner to 5-HT5 receptors decreases with increasing binding affinity. With respect to medical use, binding partners are therefore preferred whose binding affinity is so great that these can be administered in justifiable amounts in the course of an effective medical treatment as an active compound. Binding partners according to the invention are therefore preferably administered in daily doses of approximately 0.01 to 100 mg/kg of body weight and in particular of approximately 0.1 to 15 mg/kg of body weight on parenteral administration and 1 to 30 mg/kg of body weight on oral administration.

The competition experiments referred to above, with which that concentration of binding partner according to the invention is determined in vitro which displaces 50% of another comparison binding partner from the receptor binding site (IC_{50} values), offer one possibility of expressing the binding affinity. Thus the competitive inhibition of the binding of 5-CT to 5-HT5 receptors can also be evaluated to the effect that binding partners which can preferably be used according to the invention have half-maximal inhibition constants IC_{50} of less than 10^{-7} M, preferably of less than 10^{-8} M and in particular of less than 10^{-9} M.

The binding affinity of binding partners according to the invention can also be expressed by means of the inhibition constant K_i, which is in general likewise determined in vitro using competition experiments. For the binding of 5-HT5 receptors, binding partners according to the invention preferably have K_i values of less than 10^{-8} M, advantageously of l ss than 10^{-9} M and particularly preferably of less than 10^{-10} M. K_i values of compounds according to the invention are, for example, in the

range from $1 \cdot 10^{-7}$ M to $7 \cdot 10^{-7}$ M or in the range from $1 \cdot 10^{-8}$ M to $1 \cdot 10^{-7}$ M.

According to a further embodiment, binding partners according to the invention bind more selectively to 5-HT5 receptors having the advantageous binding affinities described above in relation to one or more 5-HT receptors other than 5-HT5.

According to a further embodiment, binding partners according to the invention bind more selectively to 5-HT5 receptors having the advantageous binding affinities described above in relation to all 5-HT receptors other than 5-HT5.

Binding partners are particularly advantageous which bind to 5-HT5 receptors which are expressed by glia cells and in particular by astrocytes with the affinities and selectivities described above.

According to the invention, the human receptor variant is a preferred target for the binding partners employed according to the invention.

The binding of binding partners according to the invention to 5-HT5 receptors is coupled to an effector function. Binding partners can act agonistically or antagonistically and partly agonistically and/or partly antagonistically.

Agonists are designated as compounds according to the invention which completely or partially imitate the activity of 5-HT on 5-HT5 receptors.

Agonists are designated as compounds according to the invention which can block the agonistic activity of 5-HT on 5-HT5 receptors.

According to a particular embodiment of the present invention, 5-HT5 agonists are provided as binding partners. The expression "5-HT5 agonist" designates binding partners which induce a partly to fully agonistic action. A binding partner which induces a partly agonistic action at the 5-HT5 receptor has sufficiently agonistic activity according to the invention in order to be able to be administered in justifiable amounts in the course of an efficacious medical treatment. Preferred binding partners in the context of this embodiment are those having at least 50% agonistic action. Particularly preferred binding partners are

those having at least 80% agonistic action and in particular those having essentially fully agonistic action (E_{max}).

According to a particular embodiment of th present invention, binding partners are made availabl whose binding, at least to 5-HT5 receptors of h5-HT5-transfected CHO cells, brings about a stimulation of the GTP binding to membrane-bound G proteins, an agonist-induced change in intracellular calcium levels, an induction of phospholipase C activity and/or an agonist-induced change in cAMP production. As far as the change in intracellular calcium levels is concerned, the use of binding partners which bring about an increase in intracellular calcium levels is a particular embodiment of the invention.

This embodiment also includes binding partners which are active in known animal models of cerebrovascular disorders, in particular for migraine, and/or which induce certain in vivo actions in areas of the brain, in particular genomic responses in the brain, for example the expression of transcription factors such as c-fos, c-jun, zif268 or Homer gene isoforms (Brakeman P.R. et al., Nature 386:284-288 (1997)).

Preferred binding partners are those which, also with respect to their effector function, are selective in the sense described above for 5-HT5 receptors.

According to one embodiment, 5-HT5 binding partners are low molecular weight, usually synthetic compounds.

According to a further embodiment, 5-HT5 binding partners according to the invention are 5-HT5-specific antibodies. They can be polyclonal antisera, monoclonal antibodies, antibody fragments, such as F(ab), Fc, etc., chimeric and recombinant antibodies. Such antibodies can be prepared in a manner known per se. The immunogen used can be a 5-HT5 receptor as such or antigenic fragments, as a rule fragments coupled to customary carrier proteins.

According to a further embodiment, 5-HT5 binding partners according to the invention are aptamers, i.e. nucleic acids, as a rule oligonucleotides, having sufficient affinity for 5-HT5 receptors.

Assays for the determination of binding affinities of binding partners according to the invention to 5-HT5 receptors are known in principle. This can be carried out, for example, by assessing the competitive inhibition of the binding of a comparison binding

partner to 5-HT5 receptors by the substance to be investigated. Suitable comparison binding partners are known ligands for 5-HT receptors, such as 5-HT or 5-CT or LSD. These ar expediently labeled such that their binding to 5-HT receptors can be monitored analytically using standard methods. Radioactive and optical labels are preferred. In the case of binding studies on 5-HT5 receptors, 5-CT or LSD, in particular in the form of [^{3}H]-LSD, is used according to the invention. The binding affinities can be expressed as the half-maximal inhibition constant IC_{50} or as the inhibition constant K_i. This process is preferably used for primary screening. SPA or FlashPlate technology is preferably used.

The binding to binding partners to be investigated can also be determined directly on 5-HT receptors. The inhibition constants K_i expressing binding affinity can be determined, for example, calorimetrically, i.e. by measurement of the binding energy released.

For the determination of selectivities, the binding affinity of the binding partners to be investigated for other 5-HT receptors is determined in the same manner - if appropriate using the ligands specific for the respective receptor - and the values obtained are compared.

Effector functions can also be assessed qualitatively or quantitatively both in vitro and in vivo with the aid of known functional assays.

The assessment of an agonistic activity can be based on all those effects which are caused by the binding of 5-HT to 5-HT5 receptors. It is preferred according to the invention to assess the actions on the binding of GTP to G proteins on intercellular calcium levels, on phospholipase C activity and/or on cAMP production. These processes are preferably used for secondary screening. Here too, SPA or FlashPlate technology is advantageously used.

The GTP binding to G proteins can be investigated by using a nonhydrolyzable analog of GTP, for example [$_{35}$S]GTPγS, whose binding can be investigated radiologically. This investigation is preferably carried out on membranes having a 5-HT5 receptor.

For the measurement of intracellular calcium levels, suitable calcium probes, as a rule calcium chelators, for example fluorescent compounds, such as Fura 2-acetylmethyl ester or Fluo-3-AM, can be employed. This investigation is preferably

carried out in cell cultures having a 5-HT5 receptor, in particular in individual cells.

The phospholipase C activity can be determined by means of its catalytic reactions, for example the incorporation of myoinositol, which for detection purposes is preferably radiolabeled as [^{3}H]-myoinositol, or the reaction of $PPIP_2$ to IP_3, the $PPIP_2$ preferably also being radiolabeled as [^{32}P]PIP_2. These investigations are preferably carried out on individual cells having a 5-HT5 receptor.

The cAMP production can be determined with the aid of the cAMP binding protein. This investigation is preferably carried out on individual cells having a 5-HT5 receptor.

If appropriate, the effector function is also determined, i.e. the activity of binding partners according to the invention for other 5-HT receptors. This is expediently carried out taking into consideration the binding affinities determined for 5-HT5 and other 5-HT receptors, i.e. in particular taking into consideration the selectivity.

The present invention therefore also relates to processes for the identification and characterization of binding partners according to the invention. These and further similarly suitable processes can form the basis for in vitro screening processes, with which it is possible to select from a large number of different compounds those which appear to be most promising with respect to future use. For example, it is possible by means of combinatorial chemistry to set up extensive substance banks which comprise myriads of potential active compounds. The scrutinizing of combinatorial substance libraries for substances with the desired activity can be automated. Screening robots are used for the efficient analysis of the individual assays, which are preferably organized on microtiter plates. Thus the present invention also relates to screening processes, i.e. both primary and secondary screening processes, in which preferably at least one of the processes described below is used. If a number of processes are used, this can take place at different times or at the same time on one and the same sample or on different samples of a substance to be investigated.

A particularly effective technology for carrying out processes of this type is the scintillation proximity assay, called SPA for short, which is known in the field of active compound screening. Kits and components for carrying out this assay can be obtained commercially, for example from Amersham Pharmacia Biotech. In

principle, solubilized or membrane-bound receptors are immobilized on small fluoromicrospheres containing scintillator. If, for example, a radioligand binds to the immobilized receptors, the scintillator is stimulated to emit light, as the spatial proximity between scintillator and radioligand is afforded.

A further particularly effective technology for carrying out processes of this type is the FlashPlate® technology known in the field of active compound screening. Kits and components for carrying out this assay can be obtained commercially, for example from NEN® Life Science Products. This principle is likewise based on microtiter plates (96-well or 384-well) which are coated with scintillator.

The abovementioned assays are known in principle to the person skilled in the art.

A first process according to the invention is used for the determination of the affinity and/or selectivity of binding partners for 5-HT5 receptors. For this purpose, the binding partner is brought into contact with 5-HT5 receptors and the binding affinity is determined.

A further process according to the invention relates to the determination of the activity of binding partners for 5-HT5 receptors, i.e. the determination of agonistic, partly agonistic, antagonistic and/or partly antagonistic action. For this purpose, the binding partner is brought into contact with 5-HT5 receptors and the effects produced by the binding are assessed.

According to a preferred embodiment, binding partners are subjected to a primary screening by determining their binding affinity to 5-HT5 receptors using the [^{3}H]-5-CT or [^{3}H]-LSD competition experiment described above. Those binding partners which have an inhibition constant IC_{50} in the range of 10^{-6} M or less are then subjected to a secondary screening by assessing their effector function in the manner described above, in particular with respect to GTP binding and/or the intracellular calcium levels. Finally, the binding partners selected in this way are subjected to a counter-screening for selectivity determination by determining their binding affinity to further 5-HT receptors essentially in the manner described above - but optionally using the ligands specific to the respective receptor. For example, [^{3}H]-8-hydroxydipropylaminotetralin ([^{3}H]-8-DPAT) can

b used for binding studies to 5-HT1A receptors, while 5-HT1B and 5-HT1D receptors can be investigat d using [^{3}H]-5-CT.

5-HT5 receptors are preferably made available in the form of cellular systems, i.e. in the form of membranes, cells, cell colonies, tissues or organs which carry 5-HT5 receptors. Cellular systems of this type can express 5-HT5 receptors by nature, but they can also be induced to express 5-HT5 by suitable genetic manipulation, e.g. by transfection. In the context of the preferred embodiment of the present invention concerning h5-HT5, the coding sequence described in Rees S. et al, FEBS Letters 335:242-246 (1994) can in particular be used for this (accession number X81411). Human glioma cell lines are preferred as natural cellular systems having 5-HT5 receptors. Of the h5-HT5-transfected heterologous cell lines, those are preferred which express the h5-HT5 gene. Mention may be made, for example, of h5-HT5-transfected CHO cells, h5-HT5-transfected human kidney cells, in particular h5-HT5-transfected HEK293 cells, or h5-HT5-transfected C-6 glioma cells.

For the determination of selectivity, affinity and activity of binding partners according to the invention, it is also possible to use brain tissue sections and native membranes from brain parts. If radiolabels are employed, the assessment of tissue sections is preferably carried out autoradiographically.

The efficacy of binding partners according to the invention for the treatment of migraine is preferably determined on animal models which base on mechanisms which can underlie the formation of migraine.

For example, the protein extravasation induced by binding partners according to the invention can be determined. This assay can be carried out in a manner known per se, for example by using fluorescence staining (Johnson K.W. and Phebus L.A. (1998) J. Neurosci. Methods 81: 19-24) or isotopically labeled albumin (Petty M.A. et al., (1997) Eur. J. Pharmacol. 336: 127-136). For this, the animals are treated in succession with the test compound and with [^{125}I]-albumin or fluorescent dye. The trigeminal ganglion is then electrically stimulated. The radioactivity or fluorescence accumulated in the dura mater is determined. The antimigraine activity of binding partners according to the invention is seen in the protein extravasation induced by stimulation of trigeminal ganglia.

A further model is based on the distribution of the carotid blood flow. This test is also known per se (Saxena P.R. et al., (1998) Eur. J. Pharmacol. 351: 329-339). For this, radioactive microspheres are injected into the carotid. Following a stabilization phase, the animals are treated with a test compound, and the radioactivity accumulated in the brain is determined in a micro-β-counter.

The measurement of the nitroglycerin-induced c-fos gene expression and translocation is also suitable. For this, animals are treated with nitroglycerin and the compound to be tested. The c-fos gene expression and protein translocation in the nucleus is determined using in-situ hybridization or immunohistochemically (Garcia-Ladona F.J. et al., (1994) Mol. Brain Res. 21: 75-84; Garcia-Ladona F.J. et al., (1997) J. Neurosci. Res 50: 50-61).

Further models are known from Fernandes de Lima V.M. et al. (1993) Brain Res. 614: 445-51 (Retinal Spreading depression) and from Kawahara N. et al. (1999) Exp. Neurol. 108: 27-36 (Cortical Spreading Depression).

The use according to the invention of 5-HT5 binding partners comprises, in the context of treatment, a process. In this process, an efficacious amount of one or more 5-HT5 binding partners, as a rule formulated according to pharmaceutical and veterinary medical practice, is administered to the individual to be treated, preferably a mammal, in particular a human, productive animal or pet. Whether such a treatment is indicated and in which form it has to take place, depends on the individual case and is subject to medical assessment (diagnosis), the signs, symptoms and/or malfunctions present, risks of developing certain signs, symptoms and/or malfunctions, and additionally includes further factors.

As a rule, the treatment is carried out by single or repeated daily administration, if appropriate together or in alternation with other active compounds or active compound-containing preparations, such that a daily dose of approximately 0.001 g to 10 g, preferably of approximately 0.001 g to approximately 1 g, is administered to an individual to be treated.

The invention also relates to the production of a pharmaceutical agent for the treatment of an individual, preferably a mammal, in particular a human, productive animal or pet.

The binding partners according to the invention are usually administered in the form of pharmaceutical compositions which comprise a pharmaceutically tolerable excipient with at least one binding partner according to the invention and, if appropriate, further active compounds. These compositions can be administered, for example, by the oral, rectal, transdermal, subcutaneous, intravenous, intramuscular or intranasal route.

Examples of suitable pharmaceutical formulations are solid pharmaceutical forms, such as powders, granules, tablets, pastilles, sachets, cachets, coated tablets, capsules such as hard and soft gelatin capsules, suppositories or vaginal pharmaceutical forms, semisolid pharmaceutical forms, such as ointments, creams, hydrogels, pastes or patches, and also liquid pharmaceutical forms, such as solutions, emulsions, in particular oil-in-water emulsions, suspensions, for example lotions, injection and infusion preparations, eye and ear drops. Implanted delivery devices can also be used for the administration of binding partners according to the invention. In addition, liposomes, microspheres or polymer matrices can also be used.

In the production of the compositions, binding partners according to the invention are usually mixed or diluted with an excipient. Excipients can be solid, semisolid or liquid materials which are used as a vehicle or medium for the active compound.

Suitable excipients include, for example, lactose, dextrose, sucrose, sorbitol, mannitol, starches, acacia gum, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup and methylcellulose. In addition, the formulations can comprise pharmaceutically acceptable vehicles or customary excipients, such as lubricants, for example tallow, magnesium stearate and mineral oil; wetting agents; emulsifying and suspending agents; preservatives, such as methyl and propyl hydroxybenzoates; antioxidants; antiirritants; chelating agents; coating auxiliaries; emulsion stabilizers; film-forming agents; gel-forming agents; flavor-masking agents; flavor corrigents; resins; hydrocolloids; solvents; solubilizers; neutralizing agents; permeation accelerators; pigments; quaternary ammonium compounds; refatting and superfatting agents; ointment, cream or oil bases; silicone derivatives; spreading auxiliaries; stabilizers; sterilizing agents; suppository bases; tablet excipients, such as binders, fillers, lubricants, disintegrants or coatings; propellants; drying agents; opacifying agents; thickeners; waxes; plasticizers; white oils. A relevant embodiment is based on expert knowledge, such as is presented,

for example, in Fi dler, H.P., Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete [Encyclopedia of excipients for pharmacy, cosmetics and related areas], 4th Edition, Aulendorf: ECV-Editio-Kantor-Verlag, 1996.

The present invention also relates to the use of a binding partner for 5-HT5 receptors and in particular of the abovementioned particular and preferred embodiments of binding partners according to the invention for the treatment of migrainous cerebrovascular disorders, especially for the treatment of migraine and other vascular-related headaches, in particular paroxysmal headaches of the migraine type. These include the disorders described as simple and as classical migraine without or with concomitant neurological functional disorders, true and atypical migraine, and also more specific disorders of this type, for example, associated migraine, so-called migraine equivalents, digestive migraine, ophthalmic migraine, ophthalmoplegic migraine, migraine rouge, also described as cluster headache (Horton's syndrome) and cervical migraine. Treatment is understood as meaning both prophylactic therapy, in particular prophylaxis against recurring attacks, for example as interval treatment, and treatment in the case of acute symptoms, in particular during the headache phases. Successful treatment leads to a reduction of the intensity of the symptoms, in particular of the headache, of neurological functional disorders, e.g. of visual, sensory, motor disorders and speech disorders, nausea and retching, and/or of the attack frequency. Acute treatment of migraine with binding partners is preferred. The invention in particular also relates to the use of the binding partners mentioned for the treatment of those forms of disorders mentioned above in whose formation and/or course 5-HT5 receptors are involved, i.e. disorders which are modulated by a 5-HT5 receptor activity.

The term "disorder" in the sense according to the invention describes anomalies which as a rule are regarded as pathological conditions and can reveal themselves in the form of certain signs, symptoms and/or malfunctions. The treatment according to the invention can be directed at individual disorders, say anomalies or pathological conditions, but also a number of anomalies which are causally connected with one another can be summarized to give patterns, i.e. syndromes, which can be treated according to the invention.

A treatment within the meaning according to the invention comprises not only the treatment of acute or chronic signs, symptoms and/or malfunctions but also a preventive treatment

(prophylaxis), in particular as relapse or phas prophylaxis. The treatment can be accomplished symptomatically, for example as symptom suppression. It can be carried out short-term, be accomplished medium-term, or it can also be a long-term treatment, for example in the context of maintenance therapy.

Binding partners which can be used for migraine treatment can bind to 5-HT5 with a lower, an essentially identical, or a higher affinity than to a specific receptor which is different from 5-HT5.

Binding partners for 5-HT5 receptors with respect to the use according to the invention thus in particular include those whose binding affinity for 5-HT5 receptors compared with the affinity for 5-HT1 receptors, in particular 5-HT1B and/or 5-HT1D, is so high that they are advantageously suitable for the use according to the invention. This does not necessarily presuppose a comparatively more selective binding to 5-HT5 receptors, even those selective binding partners for 5-HT5 receptors are a particular embodiment of the present invention.

For example, binding partners can be used which have a high affinity both for 5-HT5 and for 5-HT1 receptors, in particular for 5-HT1B and/or 5-HT1D. High affinity in this connection means K_i values as a rule in the range from $1 \cdot 10^{-9}$ M to $1 \cdot 10^{-6}$ M. According to a particular embodiment, in the high-affinity range usable binding partners have, relative to 5-HT receptors, a binding profile that is marked by a binding affinity to 5-HT5 which, in comparison with other binding affinities of this range is essentially identical or only slightly less. Factors of 10 or less can be advantageous.

According to a further embodiment of the invention, the selective 5-HT5 binding partners described previously are employed.

According to a particular embodiment of the present invention, binding partners are employed whose binding, at least to 5-HT5 receptors of h5-HT5-transfected CHO cells, brings about a change in the agonist-induced stimulation of the GTP binding to membrane-bound G proteins, a change in intracellular calcium levels, a change in the agonist-induced induction of phospholipase C activity and/or a change in cAMP production. As far as the change in intracellular calcium levels is regarded, the use of binding partners which bring about an increase in intracellular calcium levels is a particular embodiment of the inv ntion.

The present invention is illustrated in greater detail by means of the following examples, without being restricted thereto.

Reference Example 1

h5-HT5 receptor-expressing HEK293 cells and CHO cells

The gene coding for the human 5-HT5 receptor was isolated from human tissues in a known manner by means of 3'-5'-RT-PCR (RACE system, Boehringer Mannheim). The gene sequence was then inserted into a plasmid carrying the neomycin resistance gene (pcDNA3; Invitrogen, Germany) and amplified in E. coli according to the manufacturer's instructions. A preparation of the resulting plasmid was mixed with Lipofectamin® (Gibco Life-Sciences, Germany), and HEK293 cells were incubated with a thin layer of this transfection mixture in petri dishes (2.5 cm). The transfection mixture was then replaced by neomycin-containing culture medium. Surviving cells were further cultured in DMEM-F12 medium which was supplemented with 10% fetal calf serum, 2 mM glutamine and antibiotics (90 mg of streptomycin, 90 mg of penicillin). The cells were grown to confluence under 5% CO_2, 95% atmospheric humidity and 37°C.

h5-HT5 receptor-expressing CHO cells are obtained analogously.

Reference Example 2

Cell membrane preparation

The method used here essentially follows known methods for the preparation of cell membranes from cells (Findlay J.B.C. and Evans W.H., Biological Membranes, Practical Approach (1987)). The cells cultured according to Reference Example 1 were carefully scraped off the surface of the culture vessel and centrifuged in DMEM-F12 medium at 180xg for 10 min. The cell pellets obtained were resuspended in 5 mM tris HCl buffer containing 5 mM EDTA, 5 mM EGTA, 0.1 mM PMSF and 3 mM benzamidine (pH: 7.6; buffer A) and incubated at 4°C for 15 min. The cell suspension was homogenized (6×3s) in an Ultraturrax^R (15,000 rpm) and centrifuged at 1000×g and 4°C for 1 min. The pellet was resuspended in buffer A and, as described above, homogenized and centrifuged. The supernatants from both steps were collected and centrifuged at 40,000×g and 4°C for 20 min. The pellet was resuspended and homogenized in buffer A (1×15s). The membrane suspension was centrifuged at 40,000×g and 4°C for 20 min. The resulting pellet was resuspended in buffer A containing 10%

glycerol and 1% bovine serum albumin. Aliquots were frozen and stored at -80°C until use.

Reference Example 3

Kinetics of the saturation binding of [^{3}H]-5-CT

The methodology is essentially known (Ress S. et al., FEBS Letters 335:242-246 (1994)). Membranes obtained according to Reference Example 2 (200 µl) were incubated in a total volume of 600 µl in 100 mM tris HCl containing 1 mM EDTA (pH: 7.7; buffer B) with increasing concentrations of [^{3}H]-5-CT (96 Ci/mmol), 10 µM methiothepine being added for the determination of the specific binding, while methiothepine was not added for the determination of the total binding. The mixture was incubated at 30°C for 90 min. The samples were then filtered, a Skatron^R filtration system and GF/B filters embedded in 0.3% polyethyleneimide being used. The filters were washed at 4°C with 9 ml of buffer B. The radioactivity retained on the filters was measured by means of liquid scintillation counting, 5 ml of Ultima-Gold (Packard) being used.

Reference Example 4

[^{3}H]-5-CT binding competition

The experiments on binding competition were carried out essentially following known investigations (Rees et al., 1994). Membranes obtained according to Example 2 (200 µl) were incubated in the presence of 2 nM [^{3}H]-5-CT in a total volume of 600 µl in buffer B with increasing concentrations of selected compounds. After an incubation time of 75 min at 30°C, the samples were filtered at 4°C through GF/B filters embedded in 0.3% polyethyleneimide using buffer B. The filters were washed with 9 ml of buffer B. The radioactivity retained on the filters was determined as in Reference Example 3. The total binding was defined as that binding of the radioligand which was observed without addition of further compounds. This nonspecific binding was defined as that binding of [^{3}H]-5-CT which was observed in the presence of 10 µM methiothepine. Similar systems can also be used which, as a result of use of microtiter plates, allow a high sample throughput and secondary screening.

The saturation parameters of the [^{3}H]-5-CT binding was determined both by nonlinear regression analysis and from linear plots using the SigmaPlot software (Jandel Scientific, Germany). Competition curves were set up in which the radioactive binding is expressed

as a p rcentage proportion of the total binding. Half-maximal inhibition constants IC_{50} and Hill coefficients (n_H) were determined by means of nonlinear regression analysis.

b) Identification of h5-HT5 receptor ligands by HTS using FlashPlate technology.

96-well FlashPlates which are coated with h5-HT5 membranes can be obtained from Bio Signal Inc. (Canada). [3H]-LSD was diluted to a suitable concentration in Tris HCl buffer which contains 10 mM $MgCl_2$, 0.5 mM EDTA and 0.5% of BSA. The radio ligand solution was added to the wells (25 ml), which either contained test compound or did not contain test compound. The plates were incubated at room temperature for 180 minutes and the radioactive signal was measured using a micro β-counter (Wallac). The nonspecific binding was determined using methiothepine. [3H]-LSD has an affinity of 12 nM. With increasing binding affinity of the test compound, the radioactive signal of [3H]-LSD decreased.

Reference Example 5

Determination of the agonist-induced stimulation of [^{35}S]GTPγS binding

[^{35}S]GTPγS binding assays are known. The present assay was carried out following the previously described method of Hilf, G. and Jakobs, K.H. (Eur. J. Mol. Pharmacol. 225:245-252 (1992)). Active compound-induced changes in the [^{35}S]GTPγS binding to membranes of HEK293 cells stably transfected with the h5-HT5 receptor gene were measured (see Reference Examples 1 and 2). The cell membranes (12 µg) were incubated with 50 mM triethanolamine HCl buffer (pH: 7.5) containing 6.75mM $MgCl_2$, 150 mM NaCl, 1 mM DTT, 1 mM EDTA, 10 µM GDP and [^{35}S]GTPγS. Following a 60-minute incubation at 30°C with or without addition of the active compounds to be tested, the test mixture (100 µl) was rapidly filtered through GF-B filters using a Skatron^R filtration device. The filters were rapidly washed with 50 mM tris HCl buffer (9 ml; pH: 7.5; 4°C) containing 100 mM NaCl and 5 mM $MgCl_2$. The radioactivity retained on the filters was determined by means of scintillation spectrometry, Ultima Gold scintillation fluid being used. Similar systems which allow a high throughput and secondary screening as a result of use of microtiter plates can likewise be used.

The active compound activities were expressed as a percentage proportion of the basic binding measured in the absence of the active compound. The matching of the curves was carried out using

software for nonlinear regression analysis (SigmaPlot, Jandel Scientific, Germany) according to the general equation $E=(L\times E_{max})/(L+EC_{50})$, in which E is the fficacy, L is the ligand concentration, E_{max} is the maximum efficacy and EC_{50} is that concentration which induces 50% of the maximal efficacy.

Reference Example 6

Determination of the agonist-induced change in intracellular calcium levels

The method is known (Kao J.P.Y., Methods in Cell Biology 40:155-181 (1994)). As described in Reference Example 1, h5-HT5 receptor-expressing HEK293 cells were grown in culture vessels. The cells were carefully scraped off before they were confluent. The cells were labeled with Fura 2 by incubating at room temperature with Fura 2-acetylmethyl ester (Sigma). The cells were centrifuged at 180×g for 10 min and resuspended in DMEM-F12 medium without serum and incubated at 37°C, 5% CO_2 and 95% atmospheric humidity for 45 min.

Intracellular calcium levels were determined with a fluorescence microscope which was equipped with a suitable filter exchange system (Olympus/Hamamatsu). The fluorescence ratio (340 nm/380 nm) was determined using the Argus^R software. The intracellular calcium levels were observed for a short time in individual cells without the addition of active compounds and then 30 min after addition of the active compound to be tested. Similar systems which permitted a high throughput and secondary screening as a result of the use of microtiter plates could likewise be used.

Analogously, the modulation of intracellular Ca^{2+} levels can be assessed in the HTS. For this, h5-HT5 receptor-expression CHO cells were cultured overnight in 96-well plates (30,0000 [sic] - 80,000 cells/well). The cells were labeled for one hour using HEPES buffer containing 1 mM Fluo-3-AM, 10% of pluronic acid and 2.5 mM probencid. A test compound was added to each well. For the determination of the calcium levels, the fluorescence intensity was read off using a fluorimetrically operating plate reader (Fluorometric Imaging Plate Reader; FLIPR).

Reference Example 7

Determination of the agonist-induced phospholipase C activity

The method is essentially known (Garcia-Ladona F.J. et al., Neuroreport 4:691-694 (1993)). The cells were incubated with 0.125 µM [^{3}H]myoinositol for 24 h. Unincorporated [^{3}H]myoinositol was removed from the medium and replaced by Krebs-Henseleit buffer containing 10 mM LiCl. After incubation for 10 minutes, the active compound to be tested was added. After 45 min, the reaction was stopped by replacing the stimulation medium by distilled water. If tissue samples are used, a similar procedure is employed (Garcia-Ladona et al., 1993). The cells were frozen and stored at -80°C. The production of [^{3}H]inositol monophosphate was determined by means of known chromatographic methods. A similar method can be used with tissue miniprisms. The determination of the phospholipase C stimulation was likewise carried out in a similar manner by preparing membrane fractions, as described in Reference Example 2, and incubating with [^{32}P]PIP_2 and active compounds. In this case, the production of IP3 was determined. Known processes were also optimized in order to use systems based on microtiter plates. Commercially obtainable materials allow extension to analyses with a high throughput and the carrying-out of secondary screening.

Reference Example 8

Determination of the agonist-induced change in cAMP production

The method used is essentially known (Strada S.S. et al., Methods in Neurotransmission receptor analysis: 89-110 (1990)). Cells were incubated in culture medium without serum and antibiotics for 10 min. The medium was heated at 95°C for 15 min in order to stop the reaction. The cell samples were frozen and stored at -80°C. cAMP levels were determined using commercially obtainable kits which use the cAMP binding protein. Known processes were also optimized in order to use systems based on microtiter plates. Commercially obtainable materials allow extension to analyses with a high throughput and the carrying-out of secondary screening.

Refer nce Example 9

Tissue preparation

90 min after administration of the active compound (orally, intraperitoneally, intravenously or intracerebroventricularly), the experimental animals were decapitated. The entire brain was rapidly removed from the skull, frozen on dry ice and stored at -80°C. Rat brain sections (15 µm) were obtained in a cryostat at -20°C, applied to gelatin-coated slides and stored at -30°C until use.

Example 1

According to Reference Example 3, the binding affinity of [^{3}H]-5-CT to 5-HT5 receptors was determined. Figure 1 shows a plot of bound [^{3}H]-5-CT as a function of the [^{3}H]-5-CT concentration. A dissociation constant of K_d = 0.570 nM was determined. Depending on the clonal cell line, the receptor binding density (B) varied in a range from 900-28,000 fmol/mg of protein.

Example 2

According to Reference Example 4, the binding affinities of serotoninergic compounds were determined by means of [^{3}H]-5-CT binding competition. The following IC_{50} values were obtained:

Compound	IC_{50} [M]
R(+)-8-OH-DPAT	$6.75 \cdot 10^{-7}$
Methiothepine	$6.2 \cdot 10^{-10}$
Dihydroergotamine	$1.79 \cdot 10^{-8}$
Sumatriptan	$7.6 \cdot 10^{-6}$
Methysergide	$1.48 \cdot 10^{-7}$
R(+)-Lisuride	$8.2 \cdot 10^{-9}$
Buspirone	inactive

In a further test series, the inhibition constants K_i of the following compounds were also determined ($K_i = IC_{50}/(1+C/K_d)$), where C is the concentration of [^{3}H]-5-CT and K_d was determined according to Example 1):

Compound	K_i [M]
R(+)-8-OH-DPAT	$1.25 \cdot 10^{-7}$
5-CT	$1.44 \cdot 10^{-9}$
Dihydroergotamine	$4.05 \cdot 10^{-9}$
Sumatriptan	$8.12 \cdot 10^{-7}$
Methysergide	$1.54 \cdot 10^{-8}$

Example 3

According to Reference Example 5, the active compound-induced binding of GTP to G proteins was investigated. The coupling of 5-HT5 receptors to G proteins in HEK293 cells was evident. The typical serotoninergic agonists 5-HT and 5-CT induced an increase in the [^{35}S]GTPγS binding to the cell membranes of over 40% above the base value (see Figure 2). The 5-HT5 receptor needs GDP for the coupling to G proteins, which is mediated by agonists (see Figure 3A). The 5-HT effect was dose-dependent (see Figure 4) with an EC_{50} of 2.6 µM.

Example 4

The active compound-induced effect on intracellular calcium levels was investigated according to Reference Example 6. The stimulation of the 5-HT5 receptors with R(+)-lisuride (1 µM) in HEK293 cells induced a rise in intracellular Ca^{2+} (see Figure 5).